Effects of Bioactive Tetrapeptides on Free-Radical Processes

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Injections of epithalon and cortagen to rats decreased the content of LPO products and reduced oxidative modification of proteins, which was paralleled by suppression of antioxidant activity in rat serum and cerebral cortex.

Key Words: *epithalon; cortagen; lipid peroxidation; antioxidant activity*

Numerous studies carried out at St. Petersburg Institute of Bioregulation and Gerontology showed that epithalamin and cortexin (polypeptide preparations isolated from cattle pineal gland and cerebral cortex, respectively) exhibit antioxidant properties. The geroprotective properties and biological activity of these drugs are largely determined by their antioxidant activity. Epithalamin stimulates immunity and modulates the neuroendocrine and reproductive functions [4,6]. Cortexin is characterized by nootropic activity and stimulates reparative processes in the brain [5]. Antistress effects of these polypeptides modulating the function of the nervous system play an important role in the mechanisms of their geroprotective action.

We studied the effects of epithalon (Ala-Glu-Asp-Gly) and cortagen (Ala-Glu-Asp-Pro), short peptides synthesized on the basis of analysis of amino acid composition of these polypeptides, on the intensity of free-radical processes and antioxidant system of rat blood serum and brain.

MATERIALS AND METHODS

Experiments were carried out on 30 adult male Wistar rats (230-250 g). Epithalon and cortagen were injected intraperitoneally for 5 days in a dose of 2.5 μg/kg. Parameters of free-radical oxidation and antioxidant defense were measured in rat serum and 10% brain homogenate in phosphate buffer (pH 7.4) containing 60 mM KH_2PO_4 and 105 mM KCl. The intensity of LPO was evaluated by the level of conjugated dienes (CD) and Schiff's bases (SB). The level of oxidative modification of proteins was evaluated by the content of amino acid carbonyl derivatives in proteins after reaction with 2,4-dinitrophenylhydrasine [3]. Total antioxidant activity (TAA, expressed in arbitrary units) of the serum and brain was evaluated by riboflavin chemiluminescence and was calculated by the formula:

$$TAA=(1-PS_E/PS_C)/A,$$

where PS_E and PS_C were values of total chemiluminescence for experimental and control samples, respectively, and A was protein content in the reaction mixture [2]. Antioxidant enzymes were also measured: glutathion peroxidase [1] and SOD by inhibition NBT reduction in the presence biological material in a NADH-containing system. The reduction of NBT is associated with the release of superoxide radical and SOD inhibition; enzyme activity was expressed in arbitrary units and estimated from NADH concentration by the formula:

$$C=(E_E-E_C)/0.311,$$

where E_E and E_C are optical densities in experimental and control samples at 340 nm and 0.311 is NADH molar extinction coefficient.

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RESULTS

Epithalon and cortagen suppressed the formation of LPO products (Table 1). Epithalon was most effective in this respect and decreased the contents of primary (CD) and final (SB) LPO products in the cerebral cortex. Epithalon more intensively than cortagen inhibited LPO in the brain, causing significant changes in the levels of CD and SB. Cortagen significantly reduced CD level, but only slightly affected SB level. Both peptides selectively modulated the initial stages of LPO in the serum, which manifested in a statistically significant decrease in CD level and a trend to reduction of SB content. This dynamics of LPO products was observed for values estimated per volume of medium and by protein content (Table 1). These data also indicate that, apart from LPO, the studied peptides inhibit oxidative modification of proteins, which was most demonstrative in brain tissue. Inhibition of this modification (about 15%) in the serum was observed only under the effect of cortagen. Presumably, SB under these conditions form not only by subsequent transformation of CD, but also in the reactions of bifunctional LPO products (such as MDA) with various compounds containing free amino groups, primarily with protein amino acid residues, which leads to their oxidative modification with the formation of carbonyl groups [7]. The absence of direct evidence of involvement of free oxygen radicals in oxidative modification of the protein part of lipoprotein molecule indicates that this process can be realized through free-radical products of lipid oxidation [3]. Oxidative modification of proteins in

TABLE 1. Effects of Epithalon and Cortagen on the Content of LPO Metabolites and Oxidative Modification of Proteins (OMP) in Rat Serum and Brain ($M\pm m$)

Parameter	Control	Epithalon	Cortagen
Serum			
CD, nmol/ml	3.59±0.32 (n=8)	1.44±0.12*** (n=8)	2.20±0.40* (n=6)
CD, nmol/g protein	54.10±4.16 (n=8)	19.97±1.42*** (n=8)	30.32±5.40** (n=6)
SB, arb. units/ml	19.00±1.72 (n=8)	16.88±1.14 (n=8)	18.63±2.34 (n=6)
SB, arb. units/g protein	289±28 (n=8)	239±20 (n=8)	244±24 (n=6)
OMP, μmol protein carbonyls/ml	100.9±2.0 (n=6)	100.0±4.0 (n=6)	104.0±5.0 (n=8)
OMP, μmol protein carbonyls/mg protein	1.53±0.06 (n=6)	1.39±0.04 (n=8)	1.30±0.05* (n=6)
Protein, mg/ml	66.3±2.6 (n=8)	71.4±1.7 (n=6)	72.9±2.1 (n=6)
Brain			
CD, nmol/g tissue	40.19±2.55 (n=11)	29.13±1.62** (n=10)	29.67±1.93** (n=10)
SB, arb. units/g tissue	318.0±28.9 (n=11)	202.9±19.1** (n=10)	245.2±23.9 (n=10)
OMP, μmol protein carbonyls/mg protein	7.53±0.40 (n=8)	4.95±0.09** (n=10)	4.81±0.09** (n=9)

Note. Here and in Table 2: *p<0.05, **p<0.01, ***p<0.001 compared to the control.

TABLE 2. Effects of Epithalon and Cortagen on Activity of Antioxidant System in Rat Serum and Brain ($M\pm m$)

Parameter	Control	Epithalon	Cortagen
Serum			
TAA, arb. units/mg protein	1.74±0.15 (n=8)	1.32±0.06* (n=8)	1.34±0.09* (n=6)
Glutathion peroxidase, mmol/mg protein/min	1.17±0.07 (n=8)	1.23±0.09 (n=8)	1.09±0.06 (n=8)
SOD, arb. units/mg protein	1.25±0.24 (n=6)	1.30±0.23 (n=5)	0.67±0.17* (n=6)
Brain			
TAA, arb. units/mg protein	6.34±0.34 (n=11)	4.44±0.19*** (n=10)	5.51±0.32* (n=10)
Glutathion peroxidase, mmol/mg protein/min	35.3±1.4 (n=9)	27.00±0.06** (n=10)	29.6±1.4** (n=9)
SOD, arb. units/mg protein	46.6±2.0 (n=4)	36.5±1.4 (n=5)	39.1±2.0 (n=6)

oxidative stress precedes LPO, the protein modification products being characterized by higher stability than lipoperoxides [8,9].

Further experiments were aimed at studies of the antioxidant effects of the studied peptides. Epithalon and cortagen (0.5-5.0 μg/ml) exhibited no antioxidant activity *in vitro*, but not *in vivo*, when their effects on antioxidant activity of the serum and brain tissue were clearly seen (Table 2). Cortagen suppressed TAA in the serum, epithalon in the serum and brain. The decrease in TAA was paralleled by inhibition of antioxidant enzymes: epithalon and cortagen suppressed activity of glutathion peroxidase in the brain, while cortagen significantly reduced serum SOD activity; the enzyme activity in the brain did not change (similarly as after epithalon injection). Structural similarity of epithalon and cortagen explains their similar effects on the majority of free-radical oxidation parameters studied in our experiments.

The data suggest that the effects of epithalon and cortagen are due to suppression of the formation of LPO products and oxidative modification of proteins and compensatory reduction of TAA. It cannot be excluded that these peptides reduced production of free oxygen radicals responsible for oxidative destruction of lipids and proteins. The mechanism of this phenomenon is not yet clear. It is assumed that short peptides represent an active principle of the corresponding complex polypeptide preparations based on extracts from animal raw material, which explains the similarity of their biological effects [10]. We previously showed that the antioxidant effect of epithalamin (polypeptide preparation of the pineal gland) manifests in activation of antioxidant enzymes, including SOD [11]. The present findings indicate that the mechanisms of antioxidant effects of epithalon and cortagen differ from those of epithalamine.

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